An outstanding High School science project which received international awards and demonstrates what students can achieve*

Effect of toxic riparian weeds on the survival of aquatic invertebrates

David C. Llewellyn

Great Lakes College, Forster /Tuncurry, NSW. Correspondence: PO Box 67, Pacific Palms, 2428.
email: gillian@midcoast.com.au

ABSTRACT

In order to ascertain whether introduced weeds have an effect on naturally occurring aquatic fauna, this study assesses the toxicity of 3 introduced weeds that occur along the banks of rivers and ponds in New South Wales, on 4 aquatic invertebrates. Damsel Fly nymphs, Mud Eyes, Mayfly nymphs and Backswimmers were placed in petri dishes with minced leaves from either Crofton Weed, Camphor Laurel or Privet (tests), Black Wattle or Derris dust (comparisons) or no plant material (control). The toxicity of the weeds was determined by the lifespan of the invertebrates exposed to them. Damsel Fly nymphs were adversely affected by Camphor Laurel, Crofton Weed and Derris dust, but not the other treatments. Mud Eyes were most affected by Black Wattle and Crofton Weed, though results were variable and inconclusive. Mayfly larvae were most affected by Crofton Weed and Derris dust. Backswimmers were killed very quickly by all three test weeds and Derris dust. These results show differential toxic effects of the three weeds on different invertebrates, suggesting that the distribution and abundance of aquatic invertebrates may be significantly influenced by the presence of toxic weeds along the waterway. These results add to the understanding of negative impacts of bank disturbance which leads to weed infestation of stream banks.

Introduction

In the aquatic environment there are subtle relationships between plants and animals. Aquatic fauna relies on the presence of aquatic plants for shelter and food, while vegetation along the banks (riparian vegetation) contributes to shade, therefore affecting water temperature, provides food in the form of insects dropping from leaves as well as leaves and branches entering the water.

The vegetation along the banks also binds the soil, limiting siltation of the waterway (Billyard 1995). However, when the riparian vegetation is disturbed or cleared, river banks often become infested with weeds, introduced plants that drop their leaves into the water, or become submerged. The issue investigated in this report is whether these introduced weeds are toxic to any of the naturally occurring aquatic fauna. If so, the presence of weeds may change the balance of the fauna in the stream, and thus the balance of the food chain. This would alter the distribution and abundance of some aquatic organisms as well as other fauna higher up the food chain.

It is known that some plants can have toxic effects on animals. Indeed, many of our known pesticides are made from plant extracts; for example pyrethrum, used extensively to kill garden and household insects, is extracted from a type of daisy (Hickin 1964). Aborigines throughout northern Australia have long used plants as ichthyocides (chemicals that kill fish) in order to catch fish (Bishop *et al.* 1982). While Aborigines used native plants, the effects of many introduced plants which have infested our waterways are unknown. The aim of this experiment was to investigate the effect of three land based weeds on aquatic invertebrates in freshwater.

Three weeds were chosen for investigation.

1. **Crofton Weed *Ageratina adenophora*** (Plate 1a Appendix): This is a rapid-spreading weed that has become a nuisance along the eastern coast of Australia. It thrives in cleared areas that are free from grazing, particularly in moist areas and along creeks. The plant is originally from Mexico and is considered a weed in many countries throughout the world such

*This was a High School science project prepared while at Great Lakes College, Forster/Tuncurry in year 10 (4th year of 6 year High School) at 16 years of age. The editorial committee of the *Australian Zoologist* decided to publish this school project, as an example of what young scientists could achieve. The school teachers encouraged the auther to submit it in some competitions. It won the Australia wide 2004 University of Sydney Faculty of Science Eureka Schools Prize for Biological Sciences. It was a finalist in the BHP Billiton Science Awards, Biology and Microbiology section. It was a winner of the Intel Science Award 2004 with a prize to attend the 2005 International Scientific and Engineering Fair- Phoenix Arizona. Eight million students world wide submit projects for this fair, from which about 1500 are chosen to attend. It won 1st prize for projects related to the "Benthos", North American Benthological Society Award, and 4th prize for Environmental Sciences. The science presented was identical for all competitions, but formats were changed to suit the particular award requirements. A poster was prepared for the International Fair. The format here follows the standard for *Australian Zoologist*, and again there was no change to the text and or the science of the work. (Footnote prepared by L.Llewellyn and D. Lunney).

as India, Jamaica, United States, New Zealand and Australia. It has been declared a noxious weed in the Great Lakes area of NSW and is commonly found around local waterways (Trounce & Dyason 2003).

2. **Small-leaved Privet *Ligustrum sinense* (Plate 1b Appendix):** This species of privet was introduced from Asia and is now widespread throughout the north and central coasts of New South Wales. It invades disturbed land, especially where the original vegetation has been removed. In Australia the plant has few natural predators allowing it to flower freely and extensively. It can be found growing along the banks of many local rivers and because of this, it was decided to investigate its effects on the native aquatic invertebrates (Mowatt 2001).
3. **Camphor Laurel *Cinnamomum camphora* (Plate 1c Appendix):** This plant is native to Japan and China and is widely planted as an ornamental or shade tree. It is very common in coastal areas, often occurring along waterways, and can restrict regeneration of native rainforest species. It spreads very quickly and its wood is known to repel insects such as moths. The leaves have a very strong aroma (Harden 1990).

Two other species were used in the experiment as a basis of comparison.

1. **Black Wattle *Callicoma serratifolia* (Plate 1d Appendix):** This is a native to Australia and was used in this experiment as a control. This plant mainly occurs in and around rainforests and also is found commonly growing along the banks of creeks and rocky gullies. It was thought to have no toxic effects on invertebrates (Keith Bishop pers. comm) and therefore was used to show if the presence of plant material in the water had an effect on the invertebrates.
2. **Derris *Derris involute* (Plate 1e Appendix)** This species of Derris is found near Wingham on the mid north coast of NSW. However the commercially available "Derris" used for control of Cabbage White Butterfly larvae on cabbages was used in the experiment. This is extracted from *Derris* species. It is also manufactured in an emulsified form as "Rotenone" used as an ichthyocide. This species was chosen to show the effects of a known fish poison on the aquatic invertebrates.

A number of invertebrates were caught by dip net and the most common were used in a pilot study to determine which animals would be the best for the experiment. The toxicity of the weeds was tested on the following four species of invertebrates, identified with reference to The Insects of Australia (CSIRO 1991).

1. **Damsel Fly nymph**. Order Odonata, Sub Order Zygoptera, Super Family, Coenagrionoidea, Family, Coenagrionidae **(Fig.1).**
2. **Mud Eye.** Order Odonata, Sub Order Anisoptera, Super Family Libelluloidea, Family Corduliidae **(Fig.2)**.
3. **Mayfly nymph**. Order Ephemeroptera, Sub Order Schistonota, Super Family Baetoidea, Family Baetidae **(Fig.3)**.
4. **Backswimmer**. Order Hemiptera, Sub Order Heteroptera, Super Family Notonectoidea, Family Notonectidae, Genus *Anisops* **(Fig.4).**

Pilot Study

To ascertain which invertebrates might be sensitive to the weeds, varying numbers of six different invertebrate species were placed in petri dishes (one species to a dish) with either no weed (control), or with leaves of Camphor Laurel, Crofton Weed or another introduced plant, Coral Tree. Each dish was filled with 25mL of rain water. Survival of invertebrates was monitored over 72 hours.

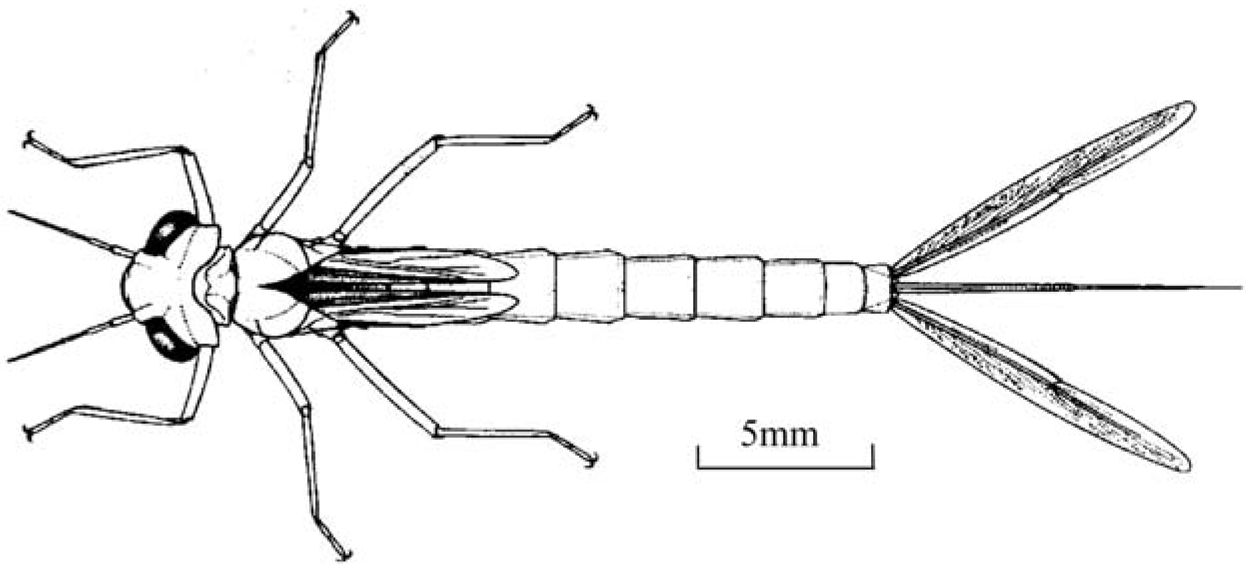

Figure 1. Order Odonata, Sub Order Zygoptera, Super Family, Coenagrionoidea, Family, Coenagrionidae. Common Name: Damsel Fly nymph. (CSIRO 1991 p. 300 Melbourne University Publishing.)

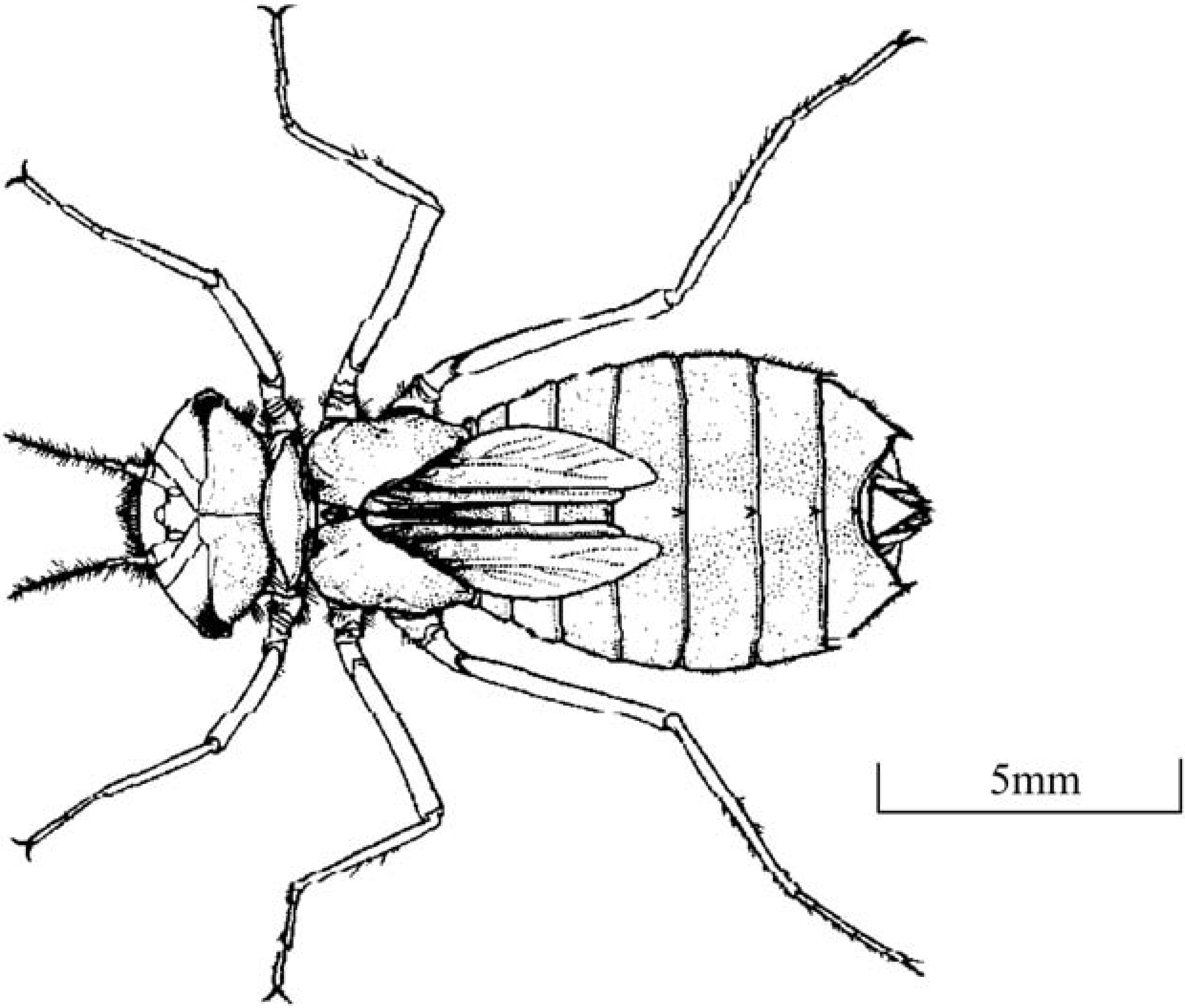

Figure 2. Order Odonata, Sub Order Anisoptera, Super Family Libelluloidea, Family Corduliidae. Common Name: Mud Eye. (CSIRO 1991 p. 301 Melbourne University Publishing.)

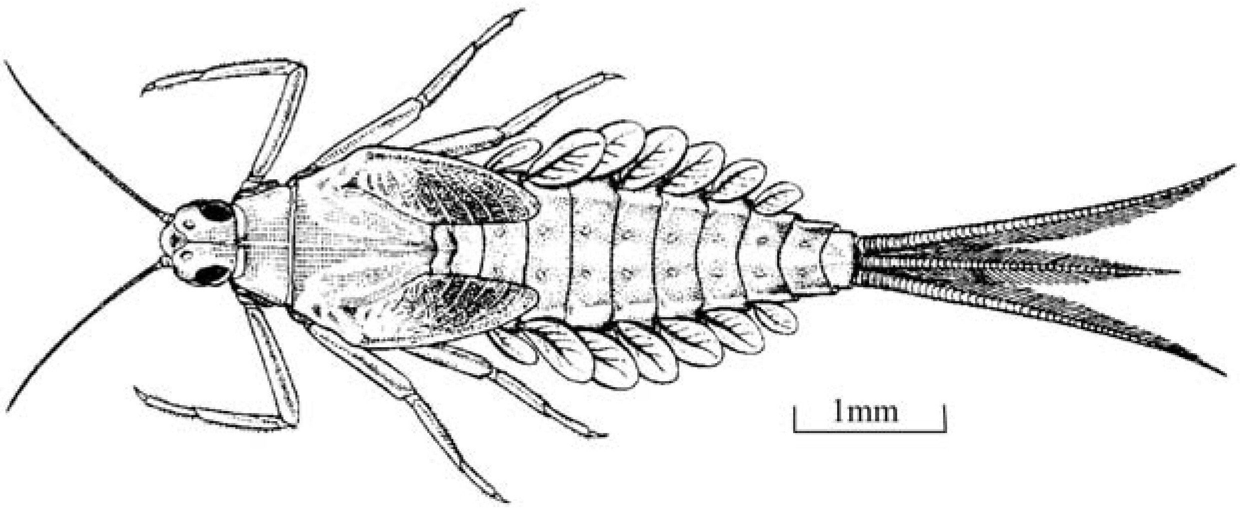

Figure 3. Order Ephemeroptera, Sub Order Schistonota, Super Family Baetoidea, Family Baetidae. Common Name: Mayfly nymph (CSIRO 1991 p. 290 Melbourne University Publishing.).

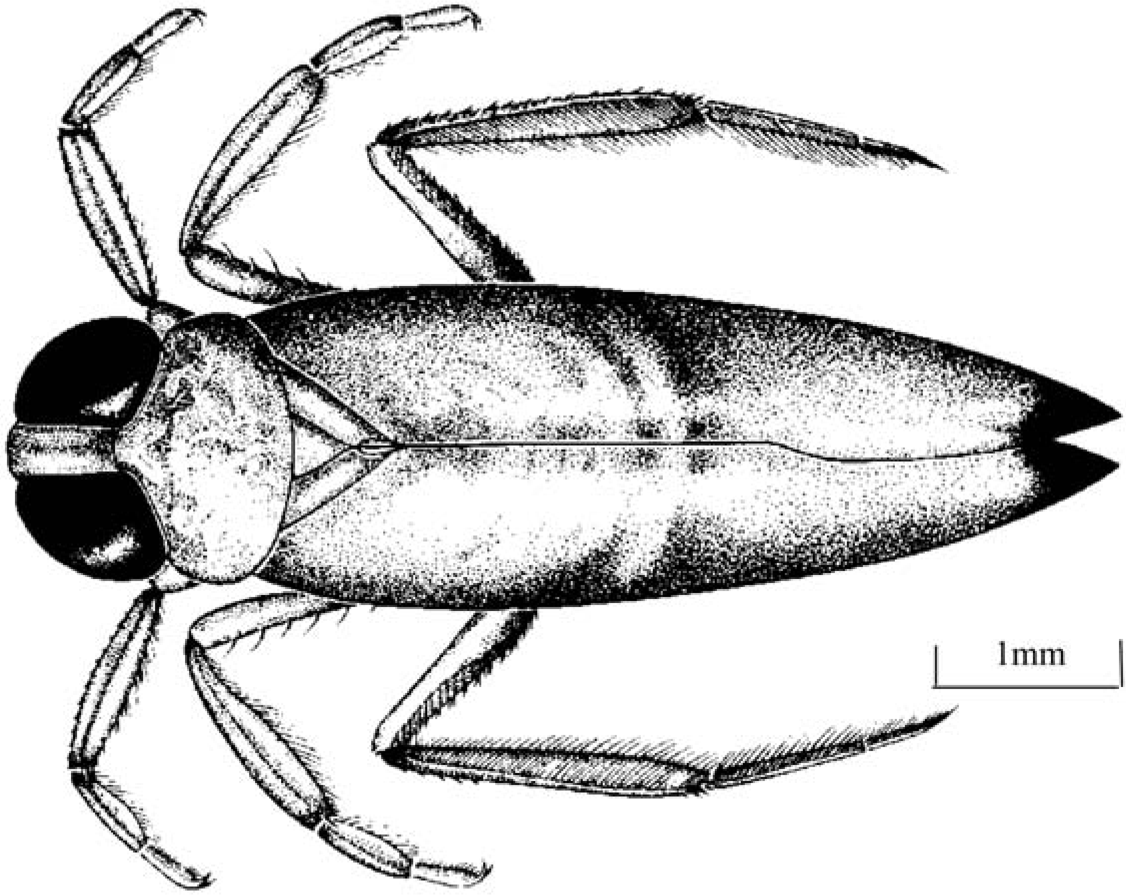

Figure 4. Order Hemiptera,. Sub Order Heteroptera, Super Family Notonectoidea, Family Notonectidae, Genus Anisops. Common Name: Backswimmer. (CSIRO 1991p. 489 Melbourne University Publishing.)

The results of this pilot experiment are shown in **Table 1**, as they helped to determine the methods used for the main experiment.

Backswimmers appeared to be very sensitive, all dying within 24 hours except in the controls. The differences between control results and test results for Backswimmers, Mud Eyes, Damsel Fly nymphs and Mayfly nymphs warranted further investigation. Water Fleas and Blood Worms were deemed unsuitable because they did not survive well in the controls. There were also insufficient numbers of Blood Worms available to continue testing with this species. Thus it was decided to investigate the 4 species of invertebrate that survived well in the control conditions, and that could be readily obtained by dip-netting in local ponds.

Insufficient Coral Tree leaves were available at this time of year, so it was decided to use another weed, Privet, instead. It was decided also to standardise the numbers of animals in each dish, and try to standardise the presentation of the leaves to facilitate finding and counting the animals. This resulted in the leaves being minced and a known quantity being tied in a mesh bag before placing it in the petri dishes. The mesh bag was used to keep the animals separate from the leaves and make them easier to observe.

Methods

1. Damsel Fly nymphs (Odonata- Zygoptera), Mud Eyes (Odonata- Anisoptera), Mayfly nymphs (Ephemeroptera) and Backswimmers (Hemiptera) were collected by dip net from local ponds.
2. 24 petri dishes were filled with 25mL of clean rainwater each. The water was measured with a measuring cylinder.
3. 5 individuals of one species of invertebrate were placed into each of 6 petri dishes.
4. Step 3 was repeated for the 3 other species.
5. 20 squares ($5cm^2$) were cut from fine netting material.
6. Leaves of Weed 1 (Camphor Laurel) were placed into a mincer and ground to fine pieces.
7. ¼ teaspoon of ground leaves was placed into each of four netting squares and tied off with a small amount of string.
8. The netting with ground leaves was placed into one dish of each species.
9. All weed was removed from the mincer and it was washed thoroughly.

Table 1. Numbers of animals surviving in petri dishes at time intervals up to 72 hours, with or without exposure to one of three introduced weeds. – means animals not counted. Air temperature = 14-15°C

Control	Time from start					
	Start (7pm)	3 Hours	15 Hours	22 Hours	24 Hours	72 Hours
Backswim.	5	5	5	5	5	5
Mud Eye	10	10	10	10	10	8
Damsel Fly	5	5	5	5	5	5
Mayfly	5	5	5	5	5	5
Blood Worm	9	9	5	5	5	5
Water Flea	5	5	3	0	0	0
Camphor Laurel	**Time from start**					
	Start (7pm)	3 Hours	15 Hours	22 Hours	24 Hours	72 Hours
Backswim.	5	4	2	0	0	0
Mud Eye	10	10	10	10	10	6
Damsel Fly	5	5	5	5	5	0
Mayfly	5	-	-	-	-	2
Blood Worm	9	7	7	6	5	1
Water Flea	5	4	3	0	0	0
Crofton Weed	**Time from start**					
	Start (7pm)	3 Hours	15 Hours	22 Hours	24 Hours	72 Hours
Backswim.	5	1	0	0	0	0
Mud Eye	10	9	9	9	8	3
Damsel Fly	5	5	5	5	5	1
Mayfly	5	-	-	3	-	1
Coral Tree	**Time from start**					
	Start (7pm)	3 Hours	15 Hours	22 Hours	24 Hours	72 Hours
Backswim.	5	0	0	0	0	0
Mud Eye	10	7	7	7	7	7
Damsel Fly	5	5	5	5	5	5
Mayfly	5	5	5	5	5	5

10. Steps 6-9 were repeated for Crofton Weed, Privet and Black Wattle.
11. ¼ level teaspoon of Derris dust (rotenone) was placed into each of the remaining pieces of netting, tying them off with string.
12. One was placed in a dish of each species (for set up see Plate 2 Appendix).
13. Survival of invertebrates in each dish was recorded approximately every 2 hours for the first 24 hours and at regular intervals up to 74 hours. Survival was determined by ability to move and observations of internal blood flow. Some individuals were recorded as "moribund" when small limb movements were detectable, but the whole animal was not mobile.
14. Survival was recorded and graphed against time for each treatment.
15. The experiment was repeated using five freshly caught individuals of each species in each dish. (Experiment 2)

Equipment List

Collecting Equipment:	Buckets and lids. Dip net.
Sorting Equipment:	Forceps. Paint brush. Ice cube tray. Shallow metal tray Microscope. Magnifying glass. Pipette.
Weed Preparation Equipment:	Mincer. Scissors. Fine netting. String. Petri dishes.
Experiment Equipment:	24 petri dishes and lids (see set up in Plate 3 Appendix). Measuring cylinder, Thermometer. Clock

Controls and Variables

Controls

In this experiment control dishes were set up exactly the same as all of the treatments except that they had no weed in them at all. Each control dish contained five individuals of the same invertebrate species and was duplicated for each of the species. These dishes, like all of the others, had 25mL of clear rainwater in them and the

dishes themselves were equal in size. The purpose of these controls was to set up a comparison with the dishes that held weeds. As long as the invertebrates survived in these dishes it was possible to conclude that those that died in the treatments died because of the treatment.

In addition. a comparison was set up with dishes containing ¼ level teaspoon of minced Black Wattle leaves. These dishes were set up exactly the same as the other petri dishes so that the only variable that was changed was the species of plant. Black Wattle is thought to be non-toxic to invertebrates (Keith Bishop pers. comm.). The reason this plant was used in the experiment was to show that the presence of a plant in the water was not killing the invertebrates. The plant matter could have been absorbing oxygen out of the water causing death by suffocation. Black Wattle was used to determine whether or not the invertebrates could live in the water with plant material in it as well. As long as the invertebrates survived in the dishes with Black Wattle, it was possible to conclude that the death of invertebrates in dishes with weeds were due to the weeds, and not just the presence of any plant matter.

Variables

There were a number of variables that had to be controlled in this experiment in order to validate the test. All the petri dishes were equal in size and depth and were all made from glass. A number of variables were relevant to water conditions. These were controlled by taking all the water used in the experiment from the same small tank which contained fresh rainwater. This meant that the temperature, salinity, pH and initial amount of oxygen in the water were all equal. Twenty five millilitres of water was measured out using a measuring cylinder and was poured into each petri dish. Once this was completed the invertebrates were sorted into species groups so that when they were placed into the dishes they would all be the same species. Five invertebrates, all of equal size, were then placed into each petri dish to standardise numbers in each dish.

Atmospheric conditions also had to be kept the same for each dish. This was achieved by placing them in the same room all subject to the same amount of light and the same temperature, humidity and air pressure. The amount of weed was a vital variable that had to be controlled. Using the same measuring spoon, ¼ level teaspoon of the particular weed was measured into each dish. The leaves of each weed were minced so that the inner cells were exposed and could release oils and other leaf products into the water. Mincing the leaves meant that each weed was presented with an equal surface area to volume ratio so that the leaf products of each weed could be released similarly. This meant that all the dishes were exposed to the same amount of weed so that toxicity could be determined. Another very important variable was the length of exposure each invertebrate had to the weed. This was controlled by putting all the weeds in at the same time and recording results for each species at the same time. If this was done too casually it would be very difficult to compare results.

Independent Variable

In this experiment the independent variable was the species of weed. The correct species was collected and this was based on past botanical knowledge and by familiarising oneself with the plant. Once collected, species were confirmed by reference to the Flora of New South Wales (Harden 1990). Leaves from one plant were kept together to avoid contamination with different species. For the second experiment the weeds had to be carefully collected to ensure that the same species was being used as the first experiment. This was especially the case with Privet because there are two different species in this area.

Dependent Variable

The variable that was measured was the length of the invertebrate's life. This was determined by their ability to move and by looking for blood flow within the body. This was the same for all of the species and was measured every two hours.

Results

The four different treatments showed differing effects on the four species of invertebrates. These results were compared with the results from the two controls for each species. All results are presented in Tables 2 and 3, and in Figures 5-12.

Damsel Fly Nymphs, Odonata- Zygoptera

In both experiments all the individuals in each of the controls survived the entire time of recording (74 hours) (Tables 2 and 3). Results for each of the treatments were similar in both experiments (Figures 5-6). This species was affected by Camphor Laurel after a period of 20 hours (Experiment 1)-25 hours (Experiment 2). After this time the numbers dropped quite rapidly until all of the individuals were dead. The animals subjected to Crofton Weed survived for approximately 24 hours (both experiments) before any mortalities were recorded. Unlike the results from the Camphor Laurel, 100 per cent mortality did not occur during the 74 hours.

Privet had no effect on this species, all animals surviving the period of the experiment.

Animals subjected to Derris dust started to die between eight hours (Experiment 1) and 20 hours (Experiment 2). The results were similar to those of Camphor Laurel. In both cases all individuals were dead at 74 hours.

Mud Eye, Odonata- Anisoptera

In this experiment all individuals in the control dishes (not Black Wattle) survived for the first 52 hours (Experiment 1, Table 2) and 40 hours (Experiment 2, Table 3). After this time different mortality rates occurred for the remaining time of recording. Although all results are shown in the graphs (Figures 7-8), meaningful comparisons can only be made while the controls are alive. During the first experiment Camphor Laurel, Privet and Derris dust had minimal effects. However both the Black Wattle control and Crofton Weed had a substantial effect.

In experiment 2 data was considered while the controls were alive (up to 40 hours). In this time Privet and Derris dust had a substantial effect on the animals survival, while the others showed similar patterns to the control.

Mayfly Nymph, Ephemeroptera

In the first experiment the controls began to die after 40

Table 2. Experiment 1 Numbers of invertebrates alive at times shown. Subscript numbers represent moribund animals (included in numbers alive). Plants and animals were placed in dishes at 0 hours and observed over 74 hours. Wa, Black Wattle; Co, control; Ca, Camphor Laurel; Cr, Crofton Weed; Pr, Privet; De, derris.

	Damsel fly nymph						Mud eye						Mayfly Nymph						Backswimmer					
Time in (h)	Wa	Co	Ca	Cr	Pr	De	Wa	Co	Ca	Cr	Pr	De	Wa	Co	Ca	Cr	Pr	De	Wa	Co	Ca	Cr	Pr	De
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	4_1	5	5	0	4	1	5
4	5	5	5	5	5	5	4	5	5	5	5	5	5	5	5	5	5	3	5	5	0	1	0	4
6	5	5	5	5	5	5	4	5	5	5	5	5	5	5	5	4	5	3	5	5	0	1	0	3
8	5	5	5	5	5	4	4	5	5	5	5	5	5	5	5	3	5	3	5	5	0	0	0	3_3
10	5	5	5	5	5	4	3	5	4	4	5	5	5	5	5	3_1	5	3	5	5	0	0	0	1_1
15	5	5	5	5	5	4	2	5	4	4	5	5	5	5	5	3_1	5	1	5	5	0	0	0	0
18	5	5	5	5	5	3_1	2	5	4	4	5	5	5	5	5	3_1	5	1_1	5	5	0	0	0	0
20	5	5	4_4	5	5	3_1	2	5	4	4	5	5	5	5	5	3_3	4	0	5	5	0	0	0	0
22	5	5	3_3	5	5	2	2	5	4	4	4	5	5	5	5	1_1	4	0	5	5	0	0	0	0
24	5	5	1_1	4	5	2	2	5	4	4	4	5	5	5	5	0	4	0	5	5	0	0	0	0
26	5	5	1_1	4	5	2	2	5	4	3	4	5	5	5	5	0	4	0	5	5	0	0	0	0
28	5	5	1_1	4	5	2	2	5	4	3	4	5	5	5	5	0	4	0	5	5	0	0	0	0
30	5	5	1_1	4	5	2	2	5	4	3	4	5	5	5	5	0	4	0	5	5	0	0	0	0
32	5	5	1_1	3	5	2_1	2	5	4	2	4	5	5	5	5	0	4_2	0	5	5	0	0	0	0
39	5	5	1_1	3	5	2_1	2	5	4	2	4	5	4	5	4	0	4_2	0	3	5	0	0	0	0
48	5	5	1_1	3	5	2_1	2	5	4	2	4	4_1	4	4	4	0	4_2	0	3	5	0	0	0	0
52	5	5	1_1	3	5	2_1	1	5	4	2	4	4_1	4	3	4	0	4_2	0	3	5	0	0	0	0
58	5	5	1_1	3	5	2_1	1	4	3	2_1	4	3	4	1	4	0	2	0	3	5	0	0	0	0
69	5	5	0	3	5	2_1	1	4	3_2	1	4	3	4	1	4	0	1	0	2	5	0	0	0	0
74	5	5	0	3	5	0	1	4	3_2	1	4	3	4	1	4	0	1	0	2	5	0	0	0	0

Table 3. Experiment 2. Numbers of invertebrates alive at times shown. Subscript numbers represent moribund animals (included in numbers alive). Plants and animals were placed in dishes at 0 hours and observed over 74 hours. Wa, Black Wattle; Co, control; Ca, Camphor Laurel; Cr, Crofton Weed; Pr, Privet; De, derris.

	Damsel fly nymph						Mud eye						Mayfly nymph						Backswimmer					
Time in (h)	Wa	Co	Ca	Cr	Pr	De	Wa	Co	Ca	Cr	Pr	De	Wa	Co	Ca	Cr	Pr	De	Wa	Co	Ca	Cr	Pr	De
0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	2_2	3	5	5_5
4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	1	3	4_4
6	5	5	5	5	5	5	5	5	5_1	5	5	5	5	5	5	5	5	5	5	5	0	1	3	2_2
9	5	5	5	5	5	5	5	5	4	5	5	4	5	5	5	5	5	5_1	5	5	0	0	3	1_1
13	5	5	5	5	5	5	5	5	4	4	5	3	5	5_1	5	3	5	4	5	5	0	0	3	0
17	5	5	5_3	5	5	5	5	5	4	4	5	3	5_1	4	4	2	5	3_2	5	5	0	0	3	0
20	5	5	5_3	5_1	5	3	5	5	4	4	5	3	4	3	3_1	2	5	1_1	5	5	0	0	1	0
23	5	5	5_3	4	5	3	4	5	4	4	5	3	4	3	3_1	1	3	0	5	5	0	0	1	0
25	5	5	3_3	4_1	5	3_1	4	5	4	4	4	3	4	3_1	3_1	1	3	0	5	5	0	0	1	0
27	5	5	3_3	4_1	5	3_1	4	5	4	4	4	3	4	2	3_1	1	3	0	5	5	0	0	1	0
30	5	5	2_2	4_1	5	2_1	4	5	4	4	3	3	4	1	1	1	3	0	5	4	0	0	1	0
33	5	5	2_2	4_1	5	2_1	4	5	4	4	2	3	3_1	1	1	1	3	0	4	4	0	0	1	0
37	5	5	2_2	4_1	5	1	4	5	4	4	2	3	3_1	1	1	1	3	0	4	4	0	0	1	0
40	5	5	2_2	3	5	1	4	5	4_1	4	2	3	3_1	1	1	1	3	0	4	3	0	0	1	0
43	5	5	0	2	5	1	2	4	3	3	1	2	2	1	1	1	2	0	3	2	0	0	1	0
47	5	5	0	2_1	5	0	2_1	4	2	3	1	1	2	1	1	1	2_1	0	3	2	0	0	0	0
52	5	5	0	2_1	5	0	2_1	4	2_1	3	1	1	2	1	1	1	2_1	0	2	2	0	0	0	0
61	5	5	0	2_1	5	0	2_1	4	2_1	3	1	1	2	1	1	1	1	0	2	1	0	0	0	0
68	5	5	0	1	5	0	1	2	1	2	1	0	0	1	1	0	1	0	2	1	0	0	0	0
74	5	5	0	1	5	0	1	2	1	1	1	0	0	1	1	0	1	0	2	1	0	0	0	0

hours (Table 2). In this time Crofton Weed and Derris dust had an effect while the other weeds did not (Figure 9).

The second experiment gives no source of comparison. The reason for this is that the controls died off quite early during recording (after 13 hours Table 3) (Figure 10).

Backswimmer, Hemiptera

In experiment 1 the controls survived throughout, with the first mortality of an individual from Black Wattle dying after 39 hours (Table 2). By this time all individuals from all of the other weeds had died. The mortality rates for each of these weeds were very high. In the first two hours all of the Camphor Laurel animals were dead (Figure 11).

The results in experiment 2 were similar (Table 3). Both the control and the Black Wattle animals had started to die before 30 hours had elapsed, but by this time all of the other animals were dead except for one individual in the Privet container which died by 47 hours. The mortality rate for the animals subjected to Camphor Laurel was very fast, all animals having died after four hours (Figure 12).

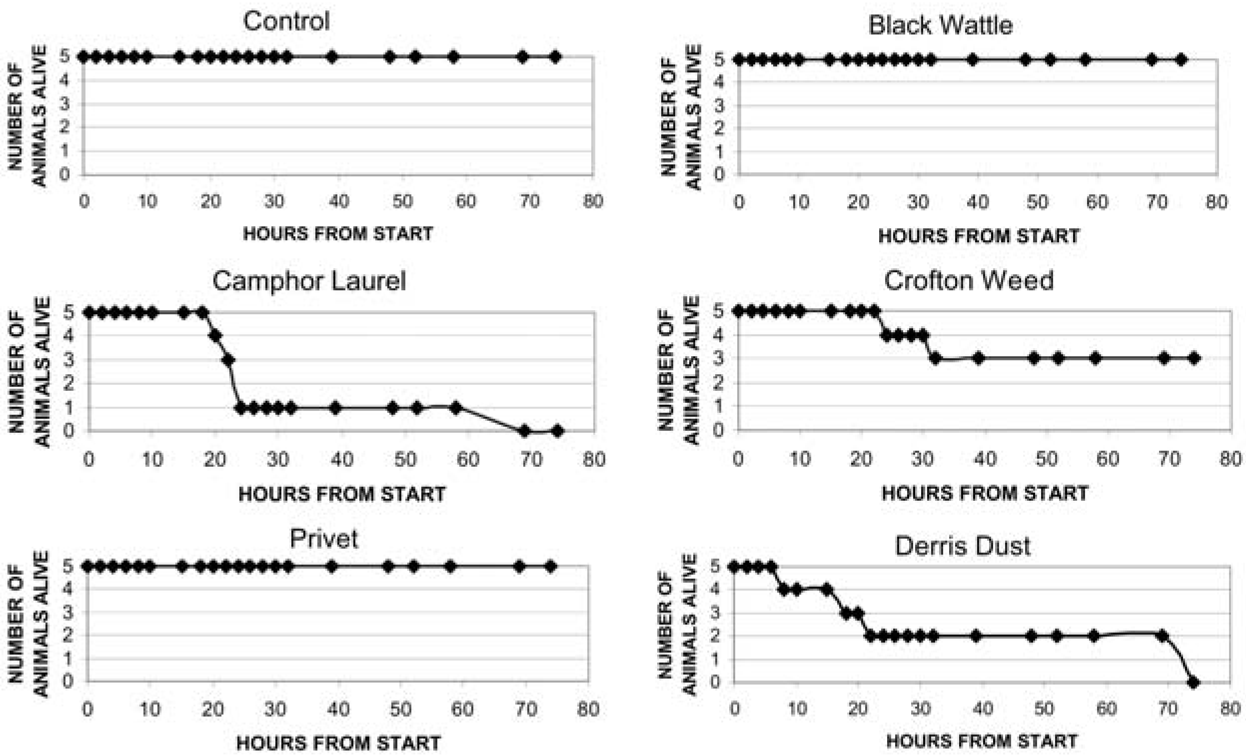

Figure 5. Experiment 1. Survival of Damsel Fly nymphs with extracts of various plants.

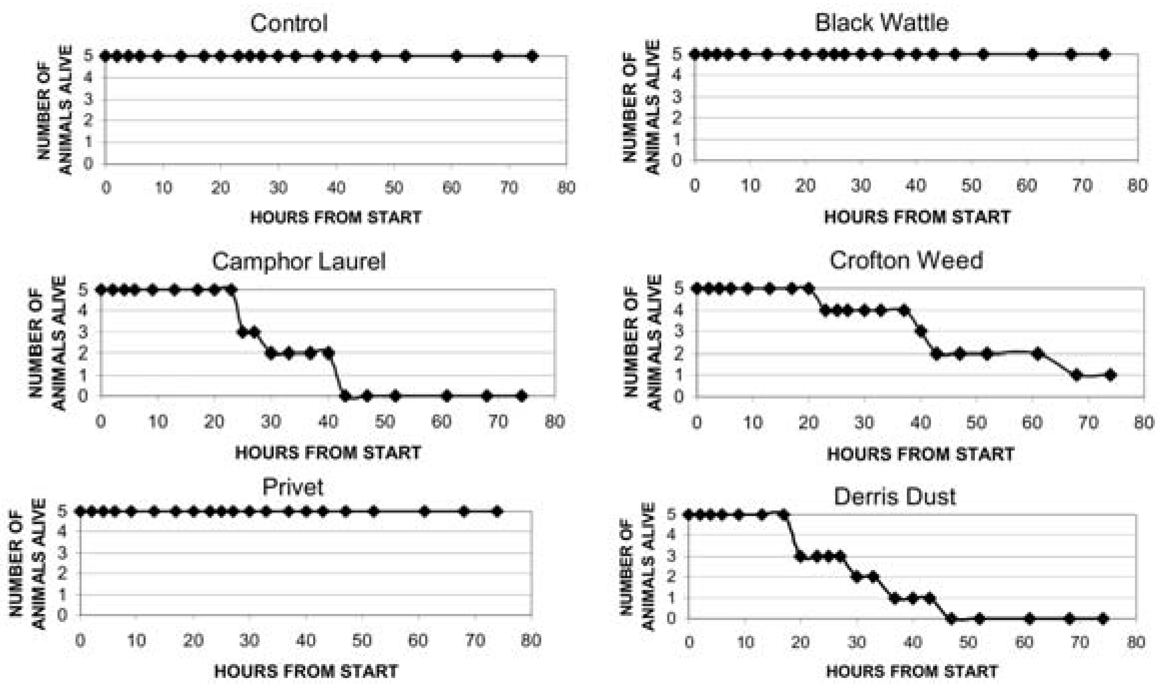

Figure 6. Experiment 2. Survival of Damsel Fly nymphs with extracts of various plants.

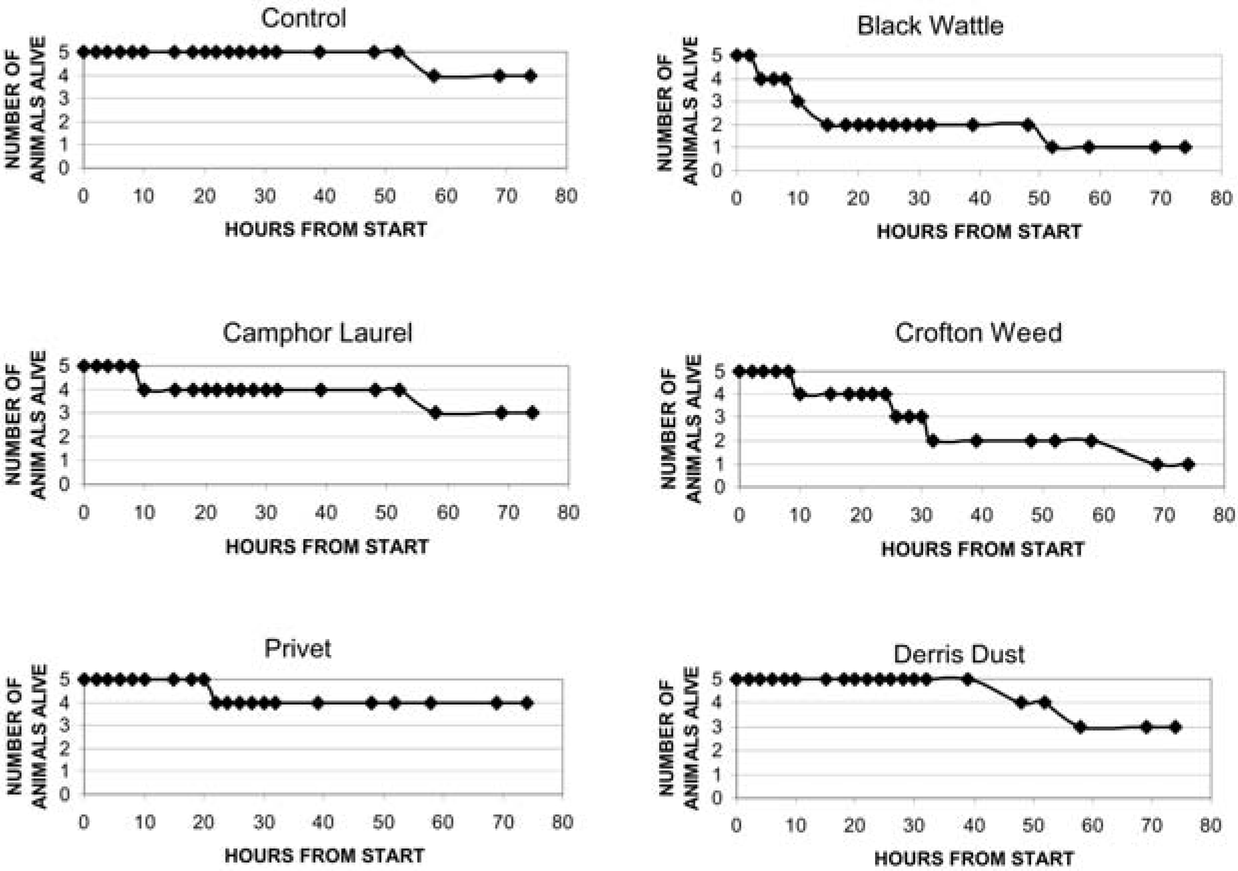

Figure 7. Experiment 1. Survival of Mud Eyes with extracts of various plants.

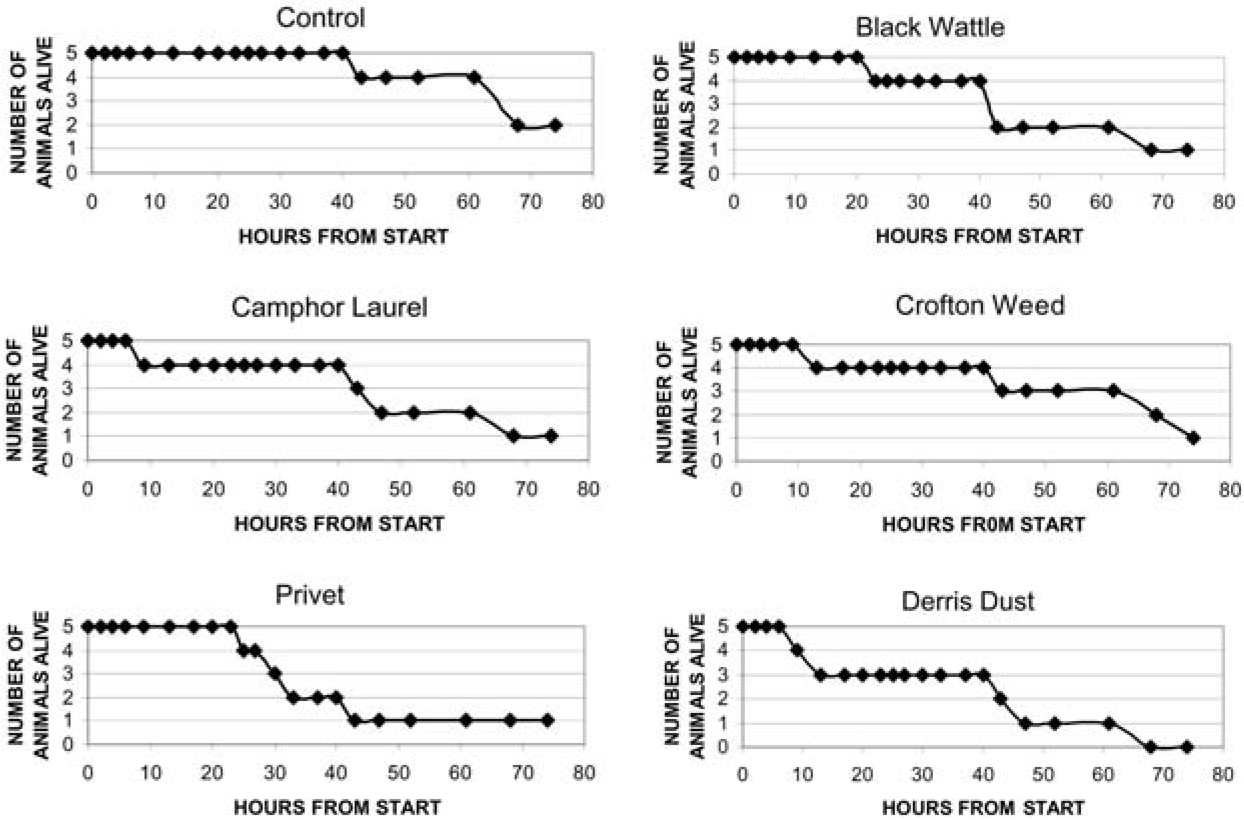

Figure 8. Experiment 2. Survival of Mud Eyes with extracts of various plants.

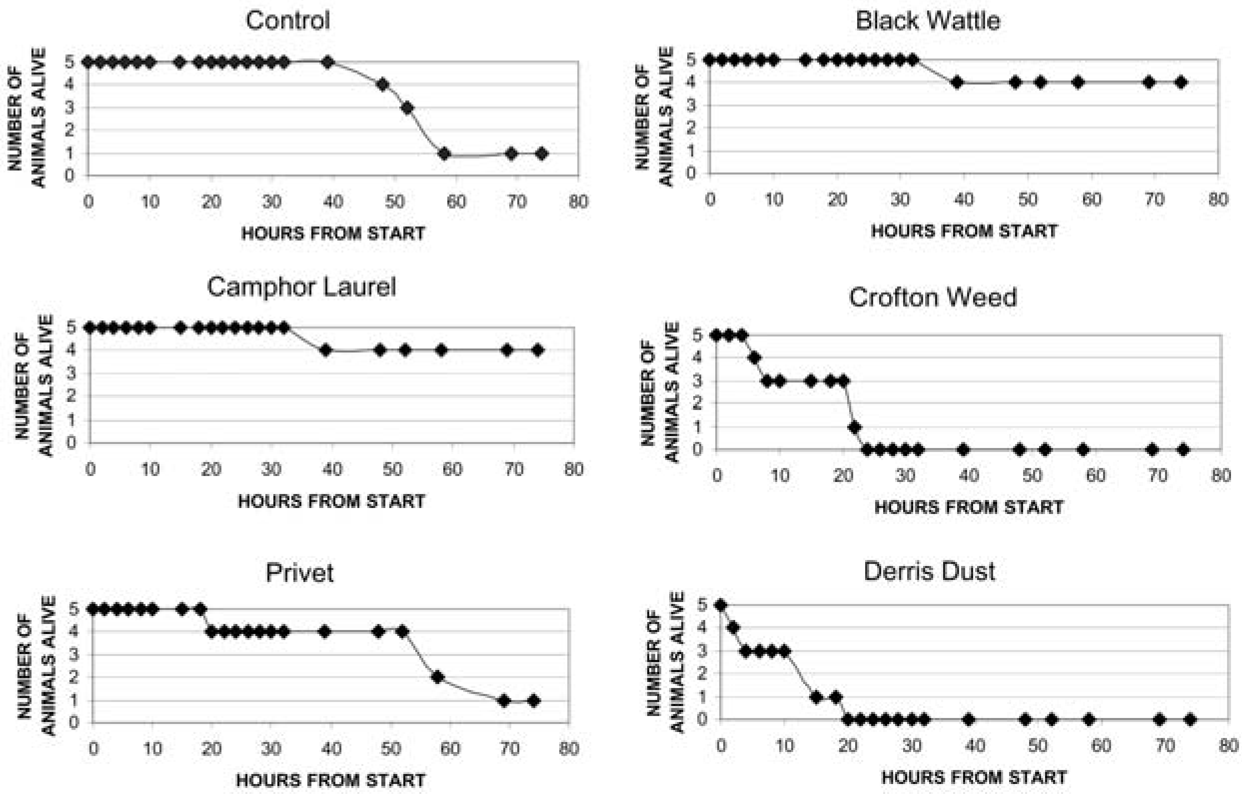

Figure 9. Experiment 1. Survival of Mayfly nymphs with extracts of various plants.

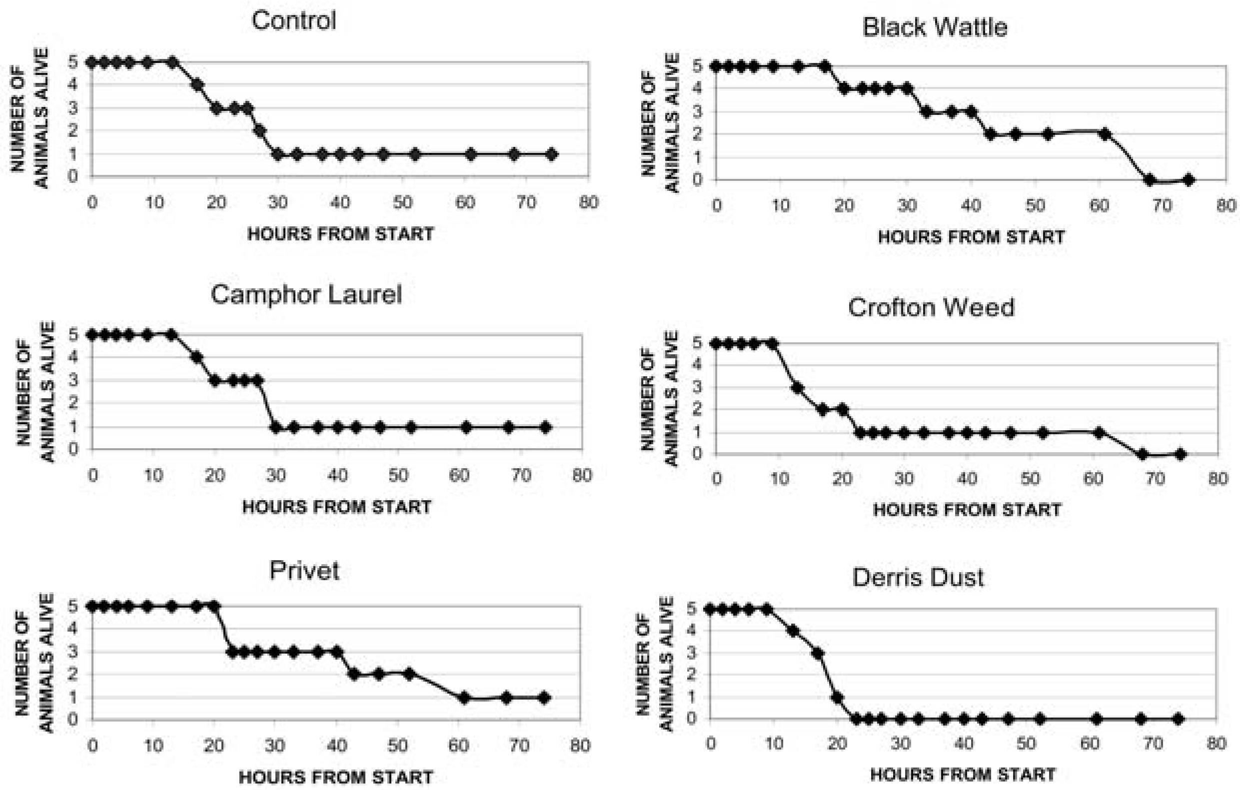

Figure 10. Experiment 2. Survival of Mayfly nymphs with extracts of various plants.

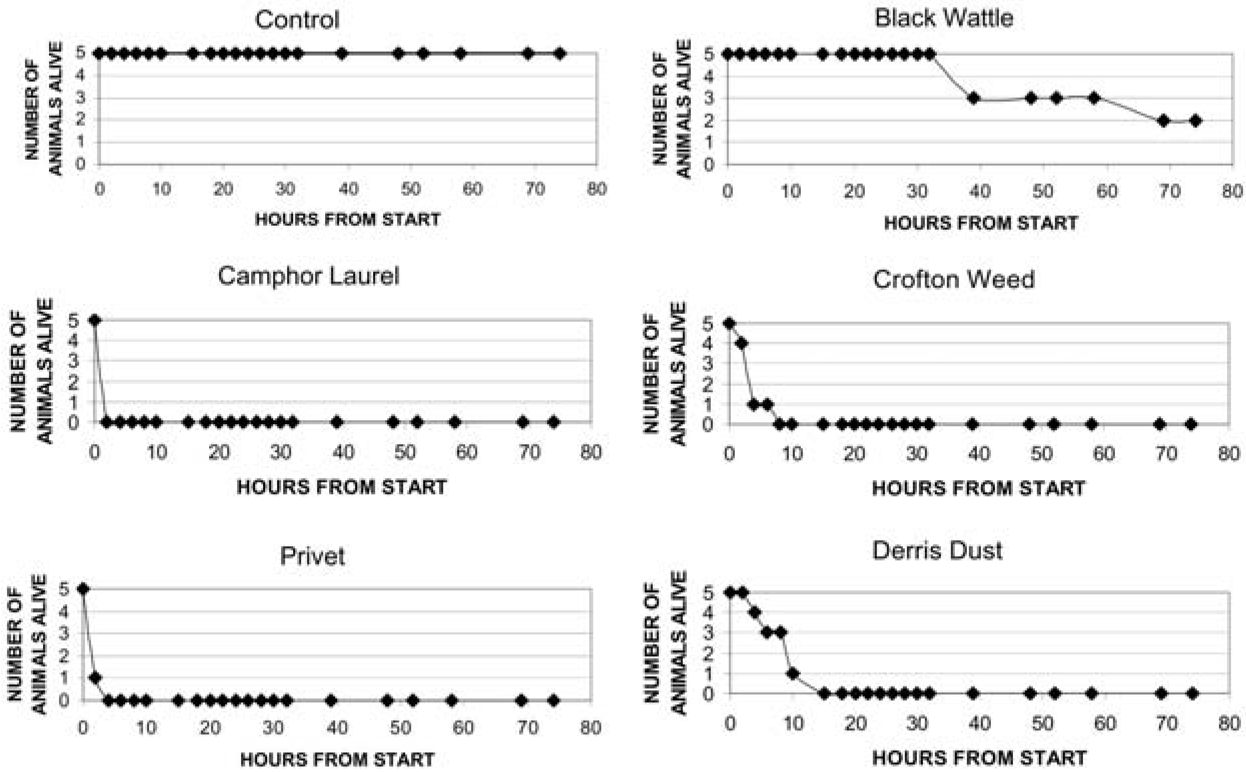

Figure 11. Experiment 1. Survival of Backswimmers with extracts of various plants.

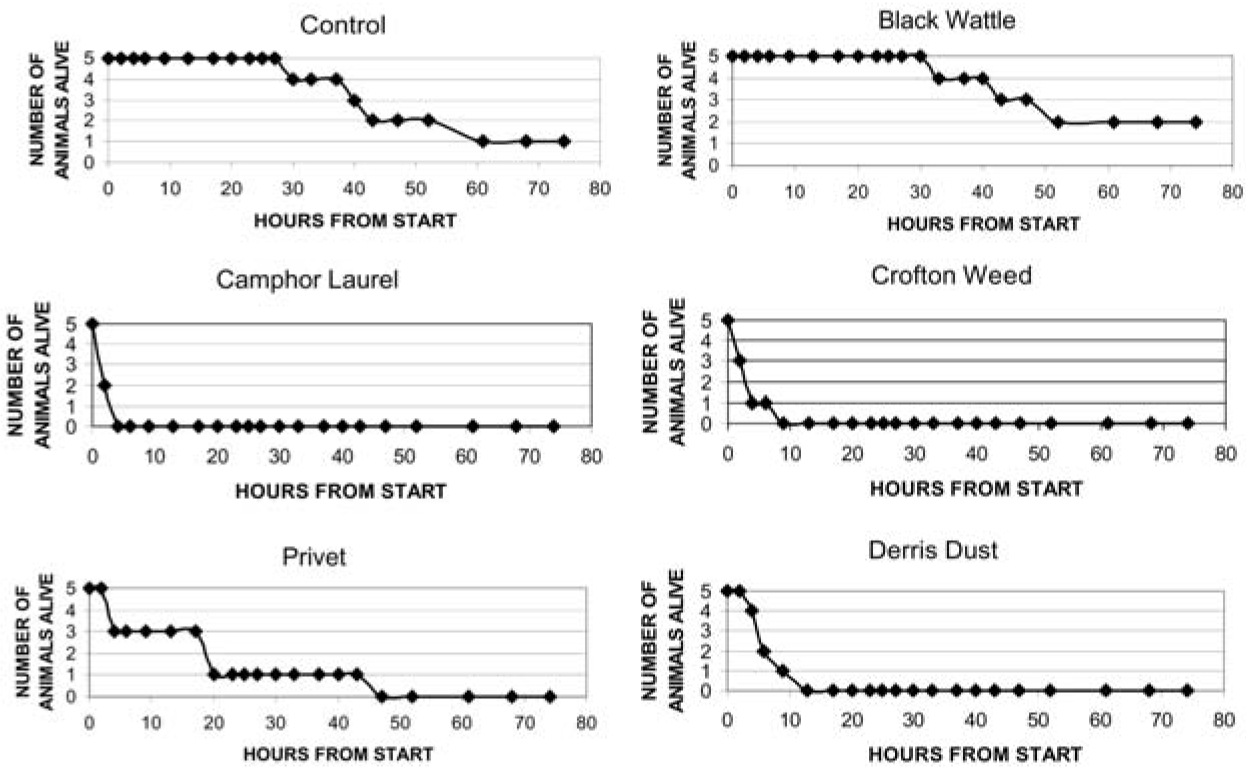

Figure 12. Experiment 2. Survival of Backswimmers with extracts of various plants.

Discussion

Controls

Survival of the animals in the control dishes, that is dishes with water and no plant material, was necessary for comparisons with the survival of animals in the test dishes. Thus, although survival in the control dishes was good, particularly in Experiment 1, comparisons with test dishes were only made during the time period when all control animals were alive.

During experiment 1, all the animals in the controls survived for the full length of the experiment (72 hr) with the exception of the Mud Eyes (Odonata- Anisoptera) and the Mayfly nymphs (Ephemeroptera). In the Mud Eye control dish only one animal died and this was after a time period of 58 hours. Experimental effects were compared before 58 hours had elapsed. By contrast four of the Mayfly nymphs died between 40 and 60 hours, indicating that this species may have been less robust under the experimental conditions than other species. It was noted during observations that this particular species was very active. This activity may have led to oxygen depletion within the petri dish. This may appear to be contradicted by the backswimmers (Hemiptera), which were also very active but survived in the control throughout the experiment. However, the backswimmers actually breathe air trapped in hairs on their body which provides for a better oxygen supply than through the water taken up by gills in the Mayfly nymphs.

Three out of the four species tested did not survive well in the control dishes during experiment 2. This is likely to be due to differing temperature conditions during the two experiments. The first experiment was conducted during cool weather with a recorded average temperature for the laboratory of 15°C. While the second experiment was taking place, considerably warmer temperatures were recorded with a maximum of 27°C. This is probably a large factor in the increased death rate of the specimens in the control dishes. The Damsel Fly nymphs (Odonata-Zygoptera), however, survived throughout the experiment suggesting that they may be more tolerant to large fluctuations in temperature along with the ability to survive under the experimental conditions.

Black Wattle

Petri dishes containing Black Wattle were set up to be comparisons to determine whether or not the invertebrates were dying as a result of having plant matter, which was presumed to be non-toxic, in the water with them. However, Black Wattle had a substantial effect on the Mud Eyes and on the Backswimmers in experiment 1. This information can be interpreted in two ways. Firstly it could be concluded that the animals in these dishes died because there was plant matter in the water with them. This could be an explanation for the results seen with the Backswimmers as all of the other weeds had already had a toxic effect and killed all the animals before the first mortality occurred in the Black Wattle dish. The other explanation is that the Black Wattle had a toxic effect on the animals. This hypothesis seems to be more accurate for the Mud Eyes as the death rate for Privet was much less than that of the Black Wattle. This would suggest that the Black Wattle itself had a toxic effect on the Mud Eyes.

In experiment 2, the animals in the Black Wattle dishes started to die at about the same time as the controls, suggesting that an external factor, probably temperature, was affecting all the dishes similarly. However, the Mud Eyes were again the exception, where those in Black Wattle died before the controls. Thus it appears that the Black Wattle was toxic to Mud Eyes but not to other species tested.

Camphor Laurel

Camphor Laurel was toxic to two of the species of invertebrates tested. In experiment 1 this species of plant did not appear to have a large effect on Mud Eyes or Mayfly nymphs though it had a very significant impact on the other two species. After 18 hours Camphor Laurel had a sudden impact on the Damsel Fly nymphs. Also it had killed all of the Backswimmers within the first two hours showing that this species is very sensitive to the presence of Camphor Laurel. These results suggest that different invertebrate species have varied tolerances to different toxins which are shown by both the rate at which two of the species died and the survival of the other two species. While the Damsel Fly nymphs have been observed to be quite robust against other weeds it was affected greatly by Camphor Laurel.

In experiment 2 similar results were observed for Backswimmers and Damsel Fly nymphs, confirming the sensitivity of these species to Camphor Laurel. The Mud Eye results were similar showing only one of the five animals dying after ten hours. Results for the Mayfly nymphs are very similar to that of the control, which displayed a high mortality rate, suggesting that the factor that killed the control animals was affecting the animals subjected to Camphor Laurel as well.

Crofton Weed

In both experiments Crofton Weed caused high death rates for all species. The rate at which the animals died varied between species showing differing sensitivities to the weed. Mayfly nymphs and Backswimmers were affected at particularly fast rates. In both cases 100 per cent mortality was observed. However the results for the Mayfly nymphs are hard to interpret in the second experiment because of high mortality in the control.

Privet

This weed proved to have differential effects on different invertebrates. In both experiments it showed no effect on Damsel Fly nymphs and had fairly rapid effects on the Backswimmers. A small effect was seen for both of the other invertebrates which varied slightly between experiments making it difficult to draw conclusions. It is interesting to note that this weed provides a large source of food for the native rainforest pigeons, so if eradication of privet is considered, it should be done gradually, alongside new rainforest plantings to replace it. This would minimise the effect on other native fauna (Recher *et al.* 1995).

The results suggest that in the case of these weeds entering a waterway, they may have an effect on the survival of some species of aquatic invertebrates while others would remain unharmed. Thus the diversity of the invertebrates in a waterway may be influenced by the presence of the weeds along that waterway.

Derris Dust

Three of the four species of invertebrate studied were significantly affected by Derris dust in both experiments, Mud Eyes showing only slight effects. The mechanism by which Derris dust kills fish does not appear to apply to Mud Eyes. It interferes with the ability of fish to uptake oxygen through the gills despite the amount of oxygen in the water (Everhart *et al.*1976). Since Mud Eyes breathe through abdominal gills it was expected that they too would die. This was not observed and warrants further investigation. Derris dust was included in this experiment because it was expected that it would have a toxic effect as it does with fish. It is interesting that all of the invertebrates survived for many hours before dying and in the case of the Mud Eyes were not affected much at all. This is in contrast to many fish which are reported to die within minutes, though some take a longer time to succumb (Everhart *et al.*1976).

It was of interest also that the Backswimmers, which uptake oxygen from air through tracheae, not gills, were also affected by Derris dust. The mechanism by which they were affected is not clear.

Water colour

During the experiment the dishes with different weeds were observed to have different coloured water in them ranging from transparent through to a dark tea colour (see Plate 2. Appendix). This is thought to have been due to the amount of oils and pigments the individual plants gave out.

Improvements

As a result of problems encountered during the pilot study, conditions in the experiments were improved. The numbers of animals in each dish were standardised due to results from the pilot study. In the pilot study the crushed weeds were placed straight into the petri dishes making it hard to find and count invertebrates. This was improved by placing the weeds into netting to constrain them and make the invertebrates clearly visible. The other thing that was determined by the pilot study was the time between recordings. This study gave a good idea of the results that could be expected and was great preparation for the experiments.

One of the problems that was experienced was that some of the controls died. This could have been due to a number of things such as temperature, amount of water in each dish and size of dish. To control these, bigger dishes could have been used with more water placed in each. Also the water could have been aerated to ensure animals were not dying from lack of oxygen.

One thing that was not measured in this experiment was the concentration of toxins in the weeds. Although the amount of weed placed in each dish was standardised, the toxins in the different weeds may have had different solubilities causing some weeds to have greater effects than others. This information could have been ascertained by sending the water away to be analysed. However, these experiments relate to the natural environment where leaves enter the waterways, and differing toxicities will be experienced depending on the solubility of the toxins in water.

Probably the biggest factor that needed to be controlled more accurately during the experiments was the temperature. This needed to be kept constant throughout both experiments to make results more comparable. Many of the differences between the results in experiments 1 and 2 could have been due to this variation in temperature. This could have been controlled by undertaking both experiments at the same time or having devices to control temperature, for example a fan, heater or air conditioner.

Application of Findings to Natural Waterways

In reality, the concentration of weeds within waterways may not reach the concentrations that were tested. However, in dry times waterways will shrink in volume and in some places form small isolated pools. Not only will these pools have a small volume of water but they may house a waterway's entire population of fauna. It is in these circumstances that the findings of this investigation are particularly relevant. These areas can be subjected to high levels of toxin concentration due to the isolation of such a water body and the small volume of water. Thus, the weeds in the riparian vegetation may have a large effect on the diversity and abundance of some species in these pools. When the river floods again, and the survivors populate the entire waterway, the distribution and abundance of the aquatic fauna may be quite different from a river in which no weeds had populated the banks.

This investigation has focussed on the effects of weeds on the invertebrate population of a waterway. However, the weeds may affect also the populations of other fauna, eg. fish and frogs. It is possible that the weeds tested in the experiment may have a direct effect on the vertebrate fauna also of a waterway. It would be interesting to investigate the effects of weeds on frog eggs and larvae with the possibility that weeds are a factor in the widespread decline of frogs.

Conclusion

In conclusion, some land based weeds are toxic to some invertebrates. Different invertebrate species show different sensitivities to each weed. Some invertebrates are affected greatly by one species of weed while they are immune to others. These results suggest that the distribution and abundance of aquatic invertebrates may be significantly influenced by the presence or absence of toxic weeds along the waterway.

The consequence of these findings is extremely important in understanding the subtle influences that plants have on the distribution of aquatic invertebrates, and therefore on other animals higher up the food chain, and adds to the understanding of the negative impacts exerted on a waterway by disturbance to riparian vegetation, which facilitates weed infestation of stream banks.

Acknowledgements

This project was undertaken as part of the Year 10 Science requirements for the School Certificate at Great Lakes College, Forster Campus. I appreciate the encouragement of my teachers Mr John Zietsch and Mr Greg Smith. I am currently a student at the Tuncurry Senior Campus of Great Lakes College.

I appreciate the input from my father, Dr Leighton Llewellyn (a biologist), who identified the invertebrates to family level. He also set up the first graph of the data, showing me how to plot the results. He counted the animals for me on a few occasions when I was asleep or at school.

My mother, Dr Gillian Courtice (also a biologist), suggested the project and provided some of the equipment to carry it out. She also read and commented on the report, before it was submitted as a school project.

I carried out the collection of the invertebrates, the pilot experiment, the setting up of the main experiments, all data collection (except on the few occasions when I asked Leighton Llewellyn to do them for me), collation of results, writing and presentation of the report. I borrowed a digital camera from a neighbour to photograph the experiment.

Thanks also to Melbourne University Publishing (as publisher) and CSIRO (as author) for permission to utilise the four drawings used in Figures 1-4 from their textbook *The Insects of Australia*.

References

Billyard, R. 1995. Fishcare- Our Freshwater Rivers and Streams. *Fishnote,* NSW Fisheries.

Bishop, K.A., Baker, L.M. and Noller, B.N. 1982. Naturally-occuring Icthyocides and a Report on *Owenia vernicosa* F. Muell. (Family Meliaceae), from the Magela Creek System, Northern Territory. *Search* 13, 150-53.

CSIRO. 1991. *The Insects of Australia,* Vol. 1. Melbourne University Press, Melbourne.

Everhart, W.H., Eipper, A.W. and Youngs, W.D. 1976. *Principles of Fishery Science*. Cornell University Press, Ithaca.

Harden, G.J. 1990. *Flora of New South Wales. Vol. 1*, UNSW Press, Sydney.

Hickin, N.E. 1964. *Household Insect Pests*. Hutchinson and Co., London.

Mowatt, J. 2001. Privet. *Agfact.* NSW Agriculture.

Recher, H.F., Date, E.M. and Ford, H.A. 1995 The biology and management of rainforest pigeons in NSW. *Species management report*. No.16, NSW National Parks and Wildlife Service.

Trounce, B. and Dyason, R. 2003. Crofton Weed. *Agfact.* NSW Agriculture.

APPENDIX I

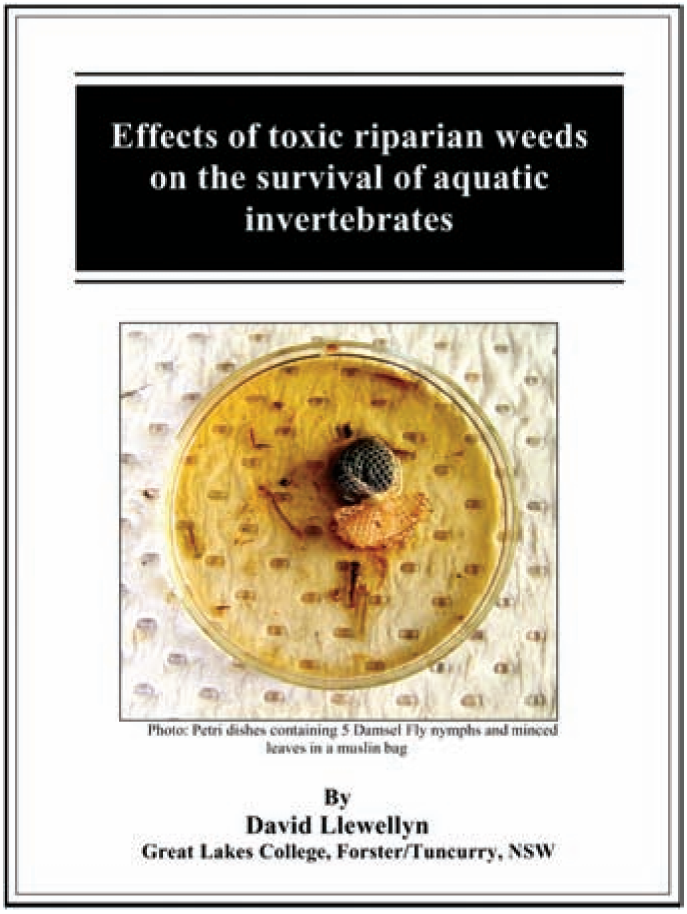
Effects of toxic riparian weeds on the survival of aquatic invertebrates

Photo: Petri dishes containing 5 Damsel Fly nymphs and minced leaves in a muslin bag

By
David Llewellyn
Great Lakes College, Forster/Tuncurry, NSW

Cover page

Thest photos all appeared in the original report. The layout here is presented as an appendix, but in each report, the photos were inserted in the text.

APPENDIX I

Plate 1a. Crofton Weed *(Ageratina adenophora)*

Plate 1b. Small-Leaved Privet *(Ligustrum sinense)*

Plate 1c. Camphor Laurel *(Cinnamomum camphora)*

Plate 1d. Black Wattle *(Callicoma serratifolia)*

APPENDIX I

Plate 1e. Derris dust

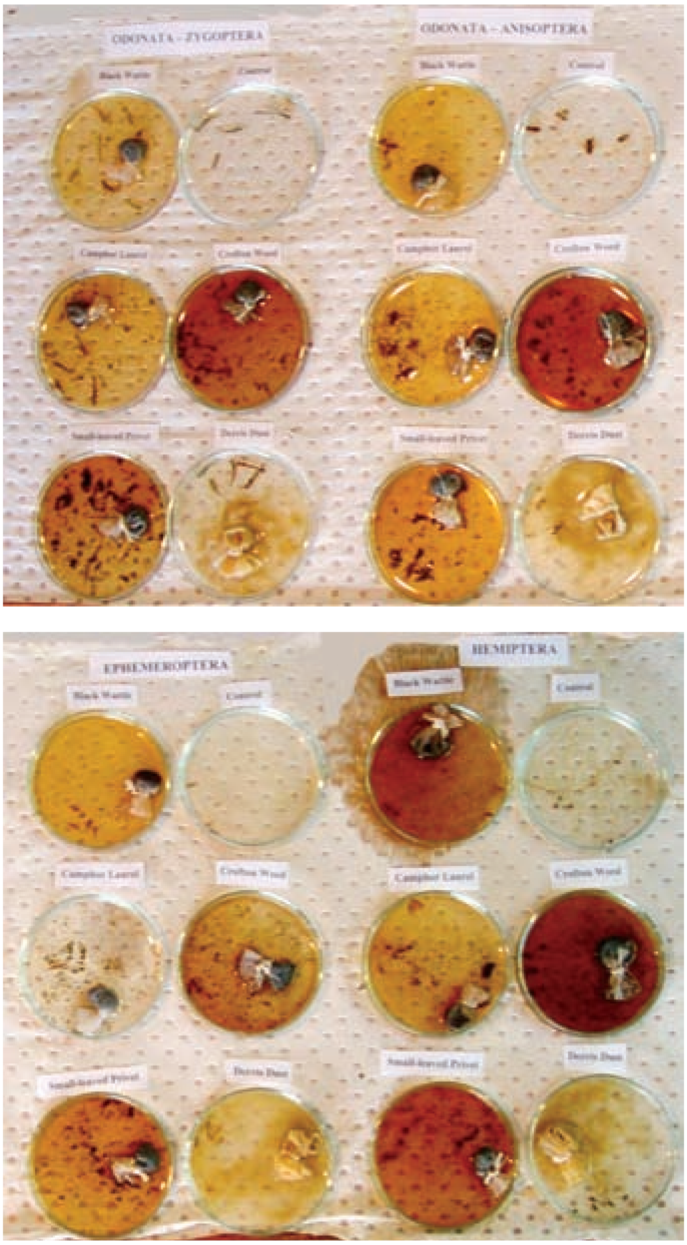

Plate 2. Layout of petri dishes in 4 groups of 6 treatments. Each group contains one species, and the treatments are as laid out below:

Black Wattle	Control
Camphor Laurel	Crofton Weed
Privet	Derris Dust

Plate 3. Layout of experiment showing equipment